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Elevated Doses of Carmustine and Mitomycin C, With Lonidamine Enhancement and Autologous Bone Marrow Transplantation in the Treatment of Advanced Colorectal Cancer: Results From a Pilot Study

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10 patients with advanced colorectal cancer were treated with elevated doses of carmustine and mitomycin C. The regimen was potentiated by lonidamine and supported by autologous bone marrow transplantation. The results of this pilot study were encouraging, with a response rate of 50% and a significantly better survival for responders versus non-responders. No appreciable toxicity of the therapy was observed. This aspect, together with the simplicity of the procedure, calls for further investigations to confirm the good therapeutic index of the treatment.

Key words: high-dose chemotherapy, autologous bone marrow transplantation, colorectal cancer, carmustine, mitomycin C, lonidamine

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INTRODUCTION

ALTHOUGH THE results obtained with fluorouracil (5-FU) are poor, the drug still represents the mainstay for the treatment of colorectal cancer (CRC) patients. Since 1975–1976, Moertel [1] and Higgins *et al.* [2] have encouraged the search for new, more rewarding therapy. In spite of this, 5-FU, enhanced by high-dose folinic acid, remains the treatment of choice for CRC notwithstanding a minimum gain in terms of response rate (RR) (20–25% to 30–35%).

Second-line anticancer agents that exhibit some activity against CRC include nitrosoureas and mitomycin C (MMC) [3, 4]. Lonidamine (LND), a derivative of indazol carboxylic acid, was recently found to potentiate the effects of some cytotoxic compounds [5, 6]. *In vitro*, the addition of LND to carmustine (BCNU) and MMC considerably decreases the concentration of these drugs required to inhibit cell proliferation by 50% in two established cell lines, LoVo and HT29, which are derived from human colon cancer (pp. 1534–1540). We designed a protocol based on elevated dose of BCNU and MMC combined with LND, with the support of autologous bone marrow transplantation (ABMT), in the treatment of advanced CRC.

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PATIENTS AND METHODS

Patients

10 patients with advanced measurable CRC were enrolled in the study, and informed consent was obtained. Patients' characteristics are reported in Table 1. Subjects were either

Table 1. Patients' characteristics

Total no. of patients	10
Age (years)	
Median	51
Range	27–60
Gender	
Male	8
Female	2
Primary tumour (*n*)	
Colon	6
Rectum	4
Site of lesions (*n*)	
Liver	4
Peritoneum	7
Lung	1
Mucinous histotype (*n*)	4

inoperable or relapsing after surgery. It was required that they had not been pretreated with alkylating agents and/or radiotherapy, but they could have received 5-FU courses. According to the other eligibility criteria, patients had to have age ≤ 60 years, negative bone scintigram, normal bone marrow cellularity, and they should not exhibit severe lung, liver, kidney and heart disease. Baseline staging was performed with radiograms, echograms and computed tomography (CT). Restaging was repeated 1 and 3 months after treatment.

Protocol

Bone marrow (600–900 ml) was explanted (time 0) from posterior iliac crest, the material obtained was filtered and stored as a normal blood transfusion unit. Immediately after removal of the marrow, patients received 6 000 000 U of α-interferon to induce hyperthermia and then they were treated with BCNU (450 mg/m^2 by infusion) and MMC (25 mg/m^2 in two injections), over 1 h. LND (900 mg) was given in three divided oral doses over 24 h (day 1). The autologous bone marrow suspension was reinfused intravenously after 48 h (day 2).

Supportive therapy and toxicologic care

A central catheter was positioned in all patients in case intensive parenteral therapy was required. Ondansetron (2 × 8 mg) was employed in the first 4 h to prevent vomiting. Following drug administration, diuresis was forced by hyperhydration (days 1–2). Reduced glutathione (GSH) was administered in continuous infusion at a daily dose of 10 g (days 1–4). Inhalational steroids were given every 2 h (days 1–2). Allopurinol and antiseptic protection (cyprofloxacine, 500 mg twice a day) were administered until there was evidence of peripheral blood cell repopulation. As a rule, no growth factors were employed. However, 3 patients, who were febrile at the time of the treatment and whose surgical wound had not completely healed, were given filgrastim (FLG) 300 μg, day 3 until evidence of leukocyte regrowth.

Haematometry was serially controlled in all patients. Special attention was payed to liver, kidney and lung function, as well as to potential signs heralding the development of a haemolytic–uraemic syndrome. 3 patients with mild coronary artery disease underwent heart monitoring upon BCNU infusion.

Statistical analysis

Median survival time was measured from chemotherapy administration. Survival curves were calculated by the Kaplan–Meier method [7] and were compared by means of the log rank test.

RESULTS

Antitumour activity

Comparative evaluation of neoplastic masses was performed through a computerised analysis of tumour areas on digital computed tomography (CT) scans. The area of each mass was measured on the slice corresponding to its equator; lesions of complex shape were measured from the manually traced perimeter. Diameters and areas were calculated in pixels, to avoid discrepancy due to incorrect pixel/cm conversion. According to usual WHO criteria, complete response (CR) implied a mass disappearance and partial response (PR) a mass reduction of ≥50%. We reported as minimal response a detectable tumour reduction less than 50%; no change in tumour mass or disease progression were considered as non-responses. In this series of patients, the response rate (RR) (one CR and four PRs) was 50%. If minimal responses are evaluated, a reduction in tumour mass was observed in 7 of 10 patients (70%). Median time to progression (TTP) was 8 months for responders and 3 months for patients exhibiting a minimal response. Overall survival of the patients is reported in Figure 1. Responders survived significantly longer than non-responders (P = 0.006; median survival 14 versus 6 months). No differences in responses were observed for colon versus rectum, for mucinous versus non-mucinous histotype, or for unpretreated versus 5-FU pretreated patients. If minimal responses were also considered, a higher effect was observed for peritoneal lesions (6 of 7 patients) than for pulmonary and hepatic lesions (0 of 1 and 2 of 4 patients, respectively).

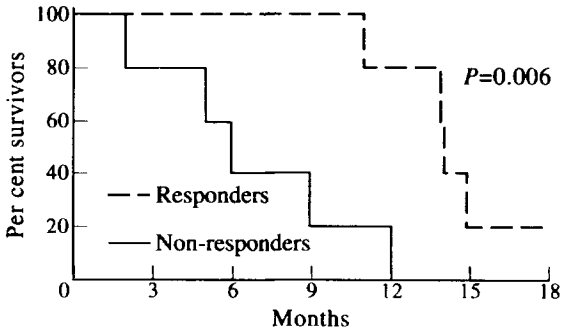

Figure 1. Overall survival of the patients

Regimen toxicity

Interferon-related fever was present in 5 of the 7 patients who were apyretic at the time of treatment. Of these, 4 were responders and 1 was a non-responder. The 2 patients without interferon-related fever were both non-responders. Apart from muscle pain due to LND, no troublesome side-effects were reported in any of our patients. Vomiting was controlled by antiemetic therapy; no mucositis, gastrointestinal distress, alopecia or significant malaise was present. As regards general toxicity, we did not observe alterations in serial blood analyses and no patient displayed microangiopathic anaemia or liver, kidney and lung failure. Heart monitoring did not evidence signs of cardiac disorders.

Figure 2 reports the haematological monitoring of platelets and white blood cells (WBC) following chemotherapy in the 7 subjects who did not receive growth factors and in the 3 who were treated with FLG. In patients without FLG treatment the occurrence of the lowest levels in WBC and platelets was reached within days 10–16 and 11–19, respectively. The mean nadir value was 787/μl (range 600–1100) for WBC and 41 900/μl (range 29 000–70 000) for platelets. The mean persistance of WBC below 1000/μl was 5.1 days (range 0–11), that of platelets below 40 000/μl was 2.0 days (range 0–6). The use of FLG induced a more prompt haematologic recovery (see Figure 2).

DISCUSSION

Although CRC is a chemoresistant tumour, high plasma levels of cytotoxic agents may improve its RR, as has been demonstrated by the effects of locoregional treatment [8, 9]. Systemic therapy with megadoses of anticancer drugs, supported by autologous bone marrow transplantation, is a treatment

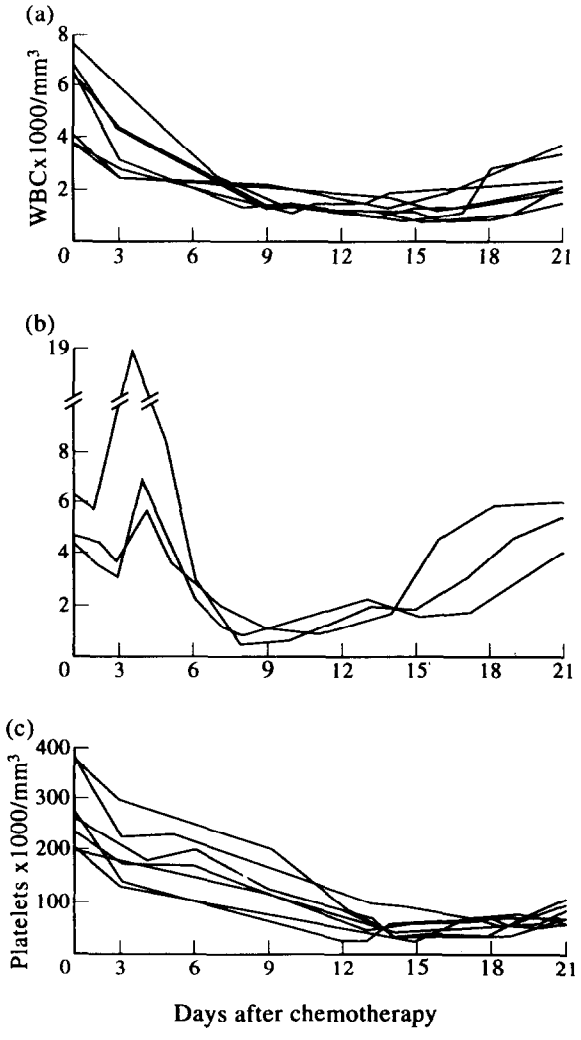

Figure 2. Peripheral blood cell dynamics of the patients. (a) WBC in subjects without filgrastim, (b) WBC in subjects treated with filgrastim, (c) platelets.

modality chiefly in use for haematological malignancies, neuroblastoma, breast cancer and other neoplasms [10–12]. Few studies have been so far carried out on CRC, most with melphalan [13] or thiotepa [14]. In these trials, responses were short and treatment toxicity was marked.

BCNU and MMC are alkylating agents that are active against CRC, although their effect is considered inferior to that of 5-FU [3, 4]. We used doses of these drugs that required bone marrow transplantation but that were not so elevated as to produce threatening toxicity. BCNU, which is commonly employed at doses of about 200 mg/m^2, was administered at a dose of 450 mg/m^2, the borderline level which should not cause lung complications [15, 16]. MMC administration was not *per se* myeloablative: in our protocol it was injected to obtain a boosting cytotoxic effect, since in combination chemotherapy MMC doses up to 30 mg/m^2 may cause renal damage [17, 18] and/or lung toxicity additional to that of BCNU [19]. MMC administrations exceeding 30–60 mg/m^2 are at risk of haemolytic–uraemic syndrome development [19–21]. Moreover, high doses of this agent may cause veno-occlusive disease of the liver [22].

Since they derive from a mucosal membrane that is in continuous contact with a number of mutagenic substances, CRC cells are supposed to have an acquired effective DNA repair capacity. BCNU is known to exert repair inhibition [23], and LND synergises the activity of alkylating agents probably through enhancement and stabilisation of nucleic acid damage [5]. Therefore, the combination of these two drugs could hopefully act on some of the CRC mechanisms of chemoresistance.

Both BCNU and MMC exert a slightly delayed myelotoxicity, especially on thrombocytogenesis. In our hands this allowed a blood cell restoration induced by the early reinfusion of bone marrow, before an extreme fall in WBC and platelets occurred. In fact, because of BCNU and MMC pharmacokinetics, a complete drug clearance within 48 h enables marrow reinfusion on day 2 [24, 25]. The procedure is also simple because CRC essentially does not metastasise to the skeletal area. It is, therefore, possible to work with the marrow instead of with peripheral stem cells, and without purging.

In our pilot study this regimen gave encouraging results, with a RR of 50%; a significantly longer survival and TTP was observed for responders than non-responders. It is obviously difficult to compare these data to previous reports, however, the survival observed by us seems better than that observed in the few studies on CRC patients treated with high doses of drugs plus ABMT.

A clinical trial with the same three drugs administered at usual doses is presently ongoing in our department. No definite conclusions can be drawn, but this combination does not seem to be very active at the plasma levels of drugs reached with ordinary chemotherapy courses.

At relapse, our patients usually exhibited a sudden widespreading of their tumour and an accelerated course of disease. This could be interpreted as a kinetic rebound to cytoreduction [26–28]. As regards the fever spike induced by interferon, responders mainly presented raised temperatures (4 of 5), while interferon-related fever was absent in 2 of 3 non-responders. It might be worthwhile to investigate whether this may be the clue to a different biological profile in responders versus non-responders.

A remarkable aspect of the study is the absence of appreciable toxicity produced by the regimen. No complications, especially renal or respiratory, or troublesome side-effects of the treatment were observed in any patient. In contrast to previous reports [29], cardiac disorders were not seen. All patients remained in excellent condition, better than that observed in outpatients treated with many of the current chemotherapy regimens. It is difficult to ascertain whether this favourable situation is due to the high dose of GSH which is known to prevent free radical damage [30, 31], and it is particularly indicated for renal safety [32, 33]. A protective antitoxic activity, not interfering with the anticancer effect, has also been documented for BCNU [34].

Economic implications of our regimen are also relevant, since it does not imply expensive organisation, technologies and supportive treatments. All the procedures can be performed within a 2-day hospitalisation period.

In conclusion, this protocol appears to be easily reproducible, safe and inexpensive. It seems to be suitable for advanced patients and as a neo-adjuvant approach. Further studies are needed to confirm these and other possibilities of application.

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